Product description

BRAC2 inherited mutations predispose carriers to various early onset cancers, including breast and ovarian. HCT116 BRCA2 -/- human colorectal carcinoma cell line was generated to study the role of BRCA2 in DNA repair, investigate the effects of BRCA2 mutations in a range of cancers, and identify additional functions of the BRCA2 gene. This includes the upregulation and effect of interferon-related genes (including APOBEC3F and APOBEC3G) when the BRAC2 gene is lost. This homozygous knockout cell line shows phenotypes consistent with previous reports, including loss of Rad51 foci in the presence of double-strand breaks, chromosomal rearrangements and elevated sensitivity to the DNA-damaging agents phleomycin and Parp1 inhibitors. This cell line has been used to show DNA damage occurs in BRCA2 knockout HCT116 cells even in the absence of exogenous genotoxic stress.

Name: HCT 116 BRCA2 -/- 46 Cell Line

Alternate name: HCT 116 BRCA2(-/-) clone 46; HCT 116 BRCA2(-/-)

Cancer: Colorectal cancer

Cancers detailed: Colorectal carcinoma

Organism: Human

Parental line: HCT 116 cell line

Tissue: Colon

Donor: 48-year-old male, Caucasian

Growth properties: Adherent

Model: Knock-out

Model description: Targeted disruption of human BRCA2 by homologous recombination

CRISPR Edited: No

Production details: Targeted disruption of the human BRCA2 locus in HCT116 cells by homologous recombination. The gene targeting construct was generated by using a recombinant adeno-associated virus (rAAV) system. The deletion was confirmed by Southern Blot and Western Blot using antibodies against the C-terminus of the BRCA2 protein. This cell line was noted as clone 46 by the originating laboratory.

Biosafety level: 1

Cellosaurus id: CVCL_VP26

HCT 116 BRCA2 -/- 46 cell line
Catalog #153531

CancerTools.org
Global Research Tools Collaborative

Contributor(s)

Inventor: Carlos Caldas
Institute: Cancer Research UK Cambridge Institute

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapour, until ready for use.

Recommended medium: McCoy's 5A medium modified, with 10% foetal bovine serum (FBS) and 2mM L-Glutamine.

Subculture: Split sub-confluent cultures (70-80%) 1:3 to 1:6 i.e. seeding at 2-4x10⁴ cells/cm² using 0.05% trypsin/EDTA solution; 5% CO_2; 37°C. Passage every 3 to 4 days.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimise exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension to a 50 mL conical tube using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
6. Wash by adding 15 - 20 mL of medium **dropwise**, while gently swirling the tube.
7. Centrifuge the cell suspension at **250 x g for 5 minutes** at room temperature.
8. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
9. Gently add required volume of culture medium and transfer to a T25 flask or 10 cm culture dish.
10. Examine the cultures after 24 hours and subculture as required.

References

- Xu et al. 2014. J Pathol. 234(3):386-97. PMID: 25043256
- Issaeva et al. 2010. Cancer Res. 70(15):6268-76. PMID: 20631063

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: HCT 116 BRCA2 -/- 46 cell line, was invented by Carlos Caldas (CancerTools.org #153531).

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